

APPENDIX 8

- J-35
6 Lacassagne, A
"Modifications Progressives De La Structure Du Conduit Tubo-Uterin, Chez Des Lapines Soumises, A Partir De La Naissance, A Des Injections Repetees D'Oestrone (Folliculine)" (*Progressive Modification of the Structure of the Tubo-Uterine Passage in Tame Female Rabbits, From Birth, With Repeated Oestrone Injections*)
CR Soc Biol, Seance November 16, 1935: 685-689
- J-35
9 Wolff, Etienne
"Sur L'Action De L'Hormone Male (Androsterone) Injectee A L'Embryon De Poulet, Production Experimentale D'Intersexues" (*On the Effect of the Male Hormone (Androsterone) Injected into the Chicken Embryo, Experimental Production of Intersexuals*)
CR Soc Biol, Seance December 13, 1935: 1312-1314
- J-35
10 Lacassagne, A
"Modifications Progressives De L'Uterus De La Souris Sous L'Action Prolongee De L'Oestrone" (*Progressive Modifications of the Mouse Uterus Under the Prolonged Effects of Oestrone*)
CR Soc Biol, Seance December 23, 1935: 1156-1158
- J-37
1 Dantchakoff, Vera
"Sur La Faculte Des Tissus Induits Par L'Hormone Male, D'Edifier De Nouvelles Structures Chez L'Embryon De Cobaye Femelle" (*On the Ability of Tissues Induced by Male Hormone to Build New Structures in the Female Guinea Pig*)
CR Soc Biol, Seance February 13, 1937: 516-521
- J-37
2 Hamilton, James B
"Masculinizing Effect of Male Hormone Substance Upon Female Reproductive Tract" - Abstract
The Anatomical Record, ed. Edward A. Boyden, Vol. 67, Supp. March 1937: 22

"Masculine changes were produced in the female reproductive tract . . . rats, rabbits, puppies and monkeys after administration of male hormone substance."
- J-37
3 Dantchakoff, Vera
"Quelques Particularites Dans Les Effects Des Hormones Sexuelles, Sur L'Embryon Du Poulet" (*Some Particularities in the Effects of Sexual Hormones on the Chicken Embryo*)
CR Soc Biol, Seance October 16, 1937: 177-180

- J-37
4 Dantchakoff, Vera
"Sur Le Sort Des Femelles Genetiques Du Poulet Traitees Par De L'Hormone Male Des Le Stade Embryonnaire" (*On the Outcome of Genetic Chicken Females Treated with Male Hormone in the Embryonic Stage*)
CR Soc Biol, Seance October 23, 1937: 275-278
- J-37
5 Raynaud, A
"Intersexualite Provoquee Chez La Souis Femelle Par Injection D'Hormone Male A La Mere En Gestation" (*Intersexuality Provoked in the Female Mouse by Injection of Male Hormone into the Mother During Gestation*)
CR Soc Biol, Seance December 12, 1937: 866-868
- J-37
6 Dantchakoff, Vera
"Sur Les Correlations De L'Ovaire Gauche, Du Rudiment Gonadique Droit Et Du Mesonephros Dans Un Embryon Femelle Testosterinise De Poulet" (*On the Correlations of the Left Ovary, the Right Gonadic Rudiment and the Mesonephros in a Testosteronized Female Chicken Embryo*)
CR Soc Biol, Seance December 18, 1937: 1191-1193
- J-38
1 Wolff, Etienne
"Sur L'Existence D'Hormones Intermediaires Susceptibles De Masculiniser Les Femelles Et De Feminiser Les Males Chez L'Embryon De Poulet" (*On the Existence of Intermediary Hormones Susceptible to Masculinize Females and to Feminize Males in the Chicken Embryo*)
CR Soc Bio De Strasbourg, Seance May 13, 1938: 420-422
- J-38
2 Dodds, EC, et al.
"Estrogenic Activity of Alkylated Stilbestrols"
Nature, Vol. 142, July 2, 1938: 34

"Diethylstilbestrol is several times more potent than oestrone and at least as potent as oestradiol."
- J-38
3 Dantchakoff, Vera
"Effects Paradoxaux D'Un Traitement Prolonge Par La Testosterone Sur L'Histogenese Sexuelle Des Femelles De Cobaye Testosterinisees Des Le Stade Embryonnaire" (*Paradoxical Effects of a Prolonged Testosterone Treatment Starting from the Embryonic Stage on the Sexual Histogenesis of Testosteronized Female Guinea Pigs*)
CR Soc Biol, Seance July 9, 1938: 1116-1119

- J-38
4 Dantchakoff, Vera
"Action De La Testosterone Et Edification Dans L'Embryon De Complexes
D'Organes Sexuels" (*Effect of Testosterone and Growth in Embryos with
Complex Sexual Organs*)
CR Soc Biol, Seance July 9, 1938: 1119-1123
- J-39
1 Winterton, WR, et al.
"Clinical Observations with Stilboestrol (Diethylstilboestrol)"
The British Medical Journal, Jan 7, 1939: 10-12

" . . . diethyl-stilboestrol . . . two and a half times as active as oestrone and to be
highly effective by mouth." p. 10

"After the treatment the nuclei of the epithelial cells became smaller and more deeply
stained as compared with the original smear." p. 11

Utero-cervical canals were noted to become larger in several patients after treatment.
- J-39
2 Burns
"Modification of Sexual Development in the Opossum by Sex Hormones"
Proc. Soc. Exp. Biol. & Med., April 1939

Estradiol injected into opossums during the last fourteen to eighteen days of
pregnancy produced stunting of the Mullerian tract.
- J-39
3 Greene, RR, Burrill, MW, and Ivy, AC
"Experimental Intersexuality: Modification of Sexual Development of the
White Rat with a Synthetic Estrogen"
Proceedings of the Society for Exp Biol and Medicine, Vol 41, May-June 1939:
169-170

" Diethylstilbestrol . . . has been shown to possess many of their functions (naturally
occurring estrogens) such as vaginal cornification, growth of the uterus . . ." p. 169

"The findings of these newborns are essentially identical with those obtained with the
natural estrogens. The external genitalia of both males and females are of the female
type . . ." p. 169

"In the female offspring the uteri are large and distended, the ovarian capsule does
not develop and the gonads are bare." p. 170

" . . . the lower vagina is partially developed. . . there is some persistence of the
Wolffian ducts and some inhibition of the lower vagina." p. 170

"From these results it is apparent that compounds other than the natural estrogens are capable of modifying embryonic sexual development in the rat." p. 170

J-39

4

Greene, Burrill, & Ivey

"Experimental Intersexuality: The Paradoxical Effects of Estrogens on the Sexual Development of the Female Rat"

The Anatomical Record, Vol. 74; May, June, July & Aug. 1939 and Supp. No. 1: 429-438

"Inhibition of normal female structures also occurs in these modified females." p. 431

"Development of the lower vagina is also inhibited in the modified females." p. 432

"... the fission process has been inhibited so that the lower vagina is only partially separated from the urethra. In some cases the extreme caudal part of the lower vagina is not formed at all." p. 432

"The females obtained from mother which were treated with high dosages of estrogens during pregnancy may be considered as having been 'masculinized' in that the development of certain structures characteristic of the male have been caused to persist or to develop." p. 437

"... there was a stimulation of certain female structures (uteri and nipples) and inhibition of other female structures (lower vagina and ovarian capsule)... there has been partial or complete preservation of the wolffian ducts." p. 438

J-39

5

Buxton, CL and Engle, ET

"Effects of the Therapeutic Use of Diethylstilbestrol"

JAMA, Vol. 113, Number 26, December 23, 1939: 2318-2320

"...more pronounced estrogenic effects when given by moth than the naturally occurring estrogens, even when the latter are injected intramuscularly." p. 2318

"...not innocuous substances..." p. 2320

"However, the observed effects in women and the abundance of data on progressive effects in animals should raise reasonable doubt in the mind of the clinician that these substances may be given with impunity." p. 2320

"...with so potent a chemical... watch for latent posttherapeutic results." p. 2320

J-40

1

Zuckerman, S

"The Histogenesis of Tissues Sensitive to Oestrogens"

Biol Revs Camb Phil Soc, No. 15 (1940): 231-271

"Massive and prolonged oestrogenic treatment may lead to considerable growth of the tube, with hyperplasia . . ." p. 233

"Excessive oestrogenic stimulation . . . of the endometrium. . . in which the normal secretory cylindrical epithelium of the cavum uteri and glands becomes transformed into stratified squamous epithelium." p. 233

". . . change occurs throughout the uterus in rats and rabbits." p. 233

". . . hypertrophies, . . . under the influence of oestrogenic stimulation. . . the connective tissue cells of the endometrium . . . Excessive stimulation may lead . . . to fibromyomatous growths in the uterus." p. 233

". . . under normal physiological conditions, oestrogenic sensitivity manifests itself histologically both in . . . the endometrium of the rat may become metaplastic. . . . Tissues derived from the Mullerian duct." p. 239

"the effects of an oestrogen on mice of either sex . . . which are concerned directly or indirectly with reproduction." p. 240

"Experiments in which the male fetuses of rats and mice are feminized by the injection of oestrogenic hormone into the pregnant mother have yielded equally important data. Greene found that the newborn male possesses a vagina . . . Raynaud found that not only the vagina, but also the uterus may in some cases develop." p. 244

"Experiment showed that it could be completely canalized by means of oestrogenic stimulation." p. 245

"When the oestrogenic stimulus becomes dominant, stratified epithelium gradually replaces the normal glandular mucosa, the change usually begins in the region closest to the original urogenital sinus. p. 249

". . . both the glandular and squamous responses to oestrogenic stimulation. . . the uterus gives the one, and the vagina the other kind." p. 253

". . . the genital and circumgenital skin in many species of monkey is sensitive to oestrogenic stimulation." p. 253

". . . the epidermis in the genital and central sexual-skin area was thicker in the

injected than in the control animals, and that the difference in thickness became increasingly greater towards the vulva and vagina." p. 255

" . . . the embryological components of the urogenital system intermix, during development. . . This fact suggests strongly that where the typical proliferative reaction of an organ like the uterus alters as a result of abnormal oestrogenic stimulation." p. 261

"The region where its mucosa meets that of the uterus is an unstable zone of transition, and an excess of oestrogenic stimulation leads to the proliferation of the vaginal type of epithelium, first in the cervix, and then, in some mammalian types, throughout the uterus." p. 261

". . . in the same way was the originally Mullerian vaginal epithelium appears to be replaced during development by cells from the urogenital sinus. This tendency is increased under the influence of oestrogenic stimulation." p. 264

J-40
2

Chen, K and Harris, P.
"The Toxicity of Stilbestrol"
Lilly Research Laboratory, Eli Lilly & Company, 1940

Quotes prior articles, i.e Gardner and Lacassagne, as well as their own study showing that Stilbestrol was 11 times more potent and in large doses resulted in the hypertrophy of the uterus, adrenals and pituitary glands, and atrophy of the uterus and thymus. Used rabbits, rats and cats.

J-41
1

Sharpey-Schafer, E and Zuckerman, S
"The Effect of Oestrogens Stimulation on the Human Prostate at Birth"
Oestrogenic Treatment of Prostate; April 1941: 431-441

Showed glandular changes similar to monkeys. Shows transplacental effect.

J-41
2

Moore, Carl
"The Influence of Hormones on the Development of the Reproductive System"
The Journal of Urology, Vol. 45, No. 6, June 1941: 869-874

" . . . duct development is conditioned by humoral substances . . ." p. 869

Hormones administered both topically and by injection into opossums.

"Treatment of females with androgens produced most unexpected results: Mullerian duct development was tremendously stimulated, with precocious uterine gland development . . . and Wolffian duct rudiments were retained usually quite atypically." p. 873

"... the inherent differences in capacity of the Mullerian ducts to react to hormones."
p. 873

"When estrogenic substances are employed on developing males and females we again meet peculiar responses. . . the female Mullerian duct is much more stimulated than the male Mullerian duct . . . the Wolffian, or male, duct responds more strongly in the female than in the male." p. 873

"Estrogens cause a tremendous hyperplasia, and metaplasia of the urogenital sinus epithelium, and in this modification, although the region is greatly stimulated, all cellular formations build up centrally rather than peripherally and the effect is a prevention of prostate differentiation." p. 873

"The observed results were obtained with pure chemical substances as hormonal agents." p. 874

"... the reproductive tract during development will respond to estrogens . . ." p. 874

"... both sets of ducts . . . will respond to . . . estrogens . . ." p. 874

J-42 Raynaud, A
1 "Modification Experimentale de la Differenciation Sexuelle des Embryons de
Souris Par Action des Hormones, Androgens et Estrogens"

DES injected in mice during 13-14th day of pregnancy showed fusion in vaginal walls and stunting of urogenital sinus.

J-42 Sklow, J.
2 "Is Human Placenta Permeable to Gonadotropic and Estrogenic Hormones?"
Proc. Soc. Exp. Biol. & Med., 49; 1942: 607

Estrogen passes through placenta.

J-42 Karnaky, K
3 "The Use of Stilbestrol for the Treatment of Threatened and Habitual Abortion and
Premature Labor: A Preliminary Report"
So. Med. J. 35; 1942: 838

Karnaky discussion: In some children, areolar area of the breast became dark in color.

- J-44
1 Greene, R
"Embryology of Sexual Structure and Hermaphroditism"
J. of Clin. Endocrinology, Vol. 4, July 1944: 335
- Brief account of embryonic development of reproductive organs, particularly the how and why in development of genital abnormalities.
- J-47
1 Roesnblum, G and Mellenhoff, E
"Preservation of the Threatened Pregnancy with Particular Reference to the Use of Diethylstilbestrol"
The West J. Surg. Obst. and Gynec. Nov. 1947: 597-603
- Studies the use of DES in threatened or habitual aborters.
- Raised four questions:
3. Can diethylstilbestrol in any way affect the glandular balance of the child in utero?
- J-47
2 Karnaky, K
"Estrogenic Tolerance in Pregnant Women"
Am. J. Obstet. and Gynecol., 53; 1947: 312
- All babies exhibited a darkening of areolae around their nipples, labia and linea albae, similar in intensity to that of their mothers, indicating that this effect of diethylstilbestrol also shared by the fetus.
- J-48
1 DeVaal, OM
"Experimentally Induced Intersexuality in Mice"
Acta Endocrinol 1, 1948: 319-338
- "For the induction of intersexuality hormone-preparations were used, i.e. substances with oestrogenic and androgenic properties." p. 320
- "The clitoris also undergoes changes . . ." p. 326
- "In one animal the ovary contained corpora lutea of pregnancy type with a Nr count of 21." p. 331
- "The same phenomenon was observed in animals 60, 70, 90, and 120 days old." p. 332
- " . . . a distinct mucification or at least a corresponding intermediate stage between cornification and mucification, that was never met with in normal mice." p. 332

J-48 Smith, OW
2 "Diethylstilbestrol in the Prevention and Treatment of Complications of Pregnancy"
Am. J. Obstet. & Gynecol., 56; 1948: 821

Raises the question of overdosage - giving estrogens in unphysiologic amounts may be toxic in fetuses.

J-48 Davis, ME and Potter, E
3 "The Response of the Human Fetal Reproductive System to the Administration of
Diethylstilbestrol and Testosterone Propionate During Early Pregnancy"
Endocrinology, 42; May 1948: 370

Important to determine in human whether therapeutic use of hormones during pregnancy may be affected by demonstrable changes in reproductive systems of male and female fetuses. 10 DES-treated fetuses examined.

Material is inadequate for conclusions concerning female fetus because only 1 was examined.

J-48 Keith, A
4 "Human Embryology and Morphology"
Edmond Arnold & Co., London, 6th Ed. 1948

"This much is certain; the epithelium that lines the developing vagina is highly susceptible to the action of estrin; so too is the epithelium which lines the urogenital sinus." p. 521

J-49 Hultquist, GT and Engfeldt, B
1 "Giant Growth of Rat Fetuses Produced Experimentally by Means of Administration
of Hormones to the Mother During Pregnancy"
Acta Endocrinol 3, 1949: 365-376

We have attempted to answer the following questions:

1. Can giant growth be produced in fetuses by administration of hormones to the mother during pregnancy? Answer is affirmative.

J-49 Burrows, H
2 "Biological Action of the Sex Hormones"
Cambridge Univ. Press, London, 1949

Estrin preparation given to guinea pigs during last 6 days of pregnancy; at birth, newborn females had cornification of vagina and swelling of the vulva.

Their mammae secreted a little colostrum and uterine horns were distended. p.706

- J-50
1 Karnacky, K
Comments on "Steroids in the Treatment of Early Pregnancy Complications"
JAMA, Vol. 142, No. 11, March 18, 1950: 778-785
- Estrogens cause abortions.
- J-50
2 Enders, RK
"Mink Production in Relation to Stilbestrol"
The Fur Journal, September/October 1950; 16(7): 4
- Total change in the ovaries of kits fed DES. Knowledge of kit failure known in 47.
DES potent hormone that mimics estrogen but behaves differently than natural
estrogen.
- J-52
1 Duraiswami, PK
"Experimental Causation of Congenital Skeletal Defects and Its Significance in
Orthopaedic Surgery"
The Journal of Bone and Joint Surgery, British Volume 34-B, February 1952: 646-
698
- "These findings lead us to conclude that, even in the pre-diabetic phase, there is an
endocrine imbalance in these women which may interfere with the normal
development of the foetus in utero." p. 658
- " . . . abnormalities induced by insulin . . ." p. 681
- "Environmental Teratogenic Factors: . . . Abnormalities of maternal hormonal mecha-
nism . . . Drugs . . . inadequate to cause abortion but perhaps sufficient to cause mal-
formations." p. 689
- J-52
2 Warren, S. and LeCompte, P.
"Infants of Diabetic Mothers"
The Pathology of Diabetes Mellitus - Ch. 28; Lea & Febiger, Philadelphia, 1952: 248-
267
- Autopsies of 50 DES exposed miscarriages, with one striking decidual reaction of
endometrium.
- J-53
1 Martinic-Dubousquet, Jacques
"Rapport Possible Entre Trois Cas D'Embryopathie et L'Administration
D'Hormones Sexuelles" (*Possible Correlation Among Three Cases of Embryo-
pathology and the Administering of Sex Hormones*)
Revue De Pathologie Generale Et Comparee, 1953: 149-160
- "Embryogenesis finishes roughly at the end of the third month but it is usually during

the first two months that most organs undergo maximal differentiation."

"... well known action of fetal or maternal hormones acting on the embryonic genital tract."

"Sexual hormones were injected in the pregnant mother before the end of the third month."

"... endogenous hormonal reaction ... we know that such actions mostly affect the genital tract."

"... possible teratologic action of hormones."

"Possible correlation between embryopathy and administration of sexual hormones: ... 3 infants, mothers of whom had received testosterone or progesterone during the first 3 months of pregnancy, showed teratological development of limbs." p. 160

J-53
2

Dieckmann, et al.

"Does the Administration of DES During Pregnancy have Therapeutic Value?"
Amer. J. of Obstet. & Gynecol, Nov. 1953; 66: 1062

Greater number of premature babies delivered to women whom stilbestrol was administered as compared to control group. Data on perinatal mortality do not indicate prophylactic administration of DES influenced favorably the fetal salvage.

J-54
1

Clarke Fraser, F, et al.

"The Experimental Production of Cleft Palate with Cortisone and Other Hormones"
Journal of Cellular and Comparative Physiology, Supp. No. 1 to Vol. 43, May, 1954: 237-259

"... until recently, hormones have received relatively little attention as teratogenic agents." p. 237

"... but little attention has been directed to effects (by sex hormones) on other parts of the embryo." p. 239

"It has been clearly established that cortisone is teratogenic in mice." p. 248

J-54
2

Berger, F

"The Pharmacological Properties of 2-Methyl-2Propyl-1, 3 Propandiol Dicarbamate (Miltown), a New Interneural Blocking Agent"
Journal of Pharm. and Exper. Therapeutics, 1954

Review of study on offspring to show state of the art drug testing.

J-54
3

Cohlan, S

"Congenital Anomalies in the Rat Produced by Prolonged Excessive Intake of

Vitamin A During Pregnancy"
Pediatrics, 1954; 13: 566

Many investigations in experimental embryology demonstrated that multitude of agents acting on fetal environment could evoke drastic teratologic changes.

In last 2 decades, evidence indicates various specific disturbances of mammalian fetal environment results in predictable congenital malformations.

J-55 Raynaud, A
1 "Frequence Et Repartition Des Malformations Mammaires Chez Les Foetus De Souris Ayant Recu Une Injection D'Hormone Oestrogene"
(Frequency of the Mammary Malformations in Female Fetuses from Mice Having Received Injection of Estradiol Dipropionate during Pregnancy)
CR Soc Biol, Seance June 25, 1955: 1229-1236

"There were various mammary malformations in the fetuses from mother treated with estradiol propionate."

" . . . a) each dose of e.d. . . . injected in a pregnant mouse provoked modifications of the development of mammary anlagen in fetuses; b) the frequency of these mammary malformation in fetuses increased with the dose of e.d. given to the pregnant mother . . ."

" . . . estradiol . . . has a strong teratogenic action on . . . fetuses. The frequency of these malformations increases with the dosage of estrogen hormone injected into the mother . . . These results draw again attention to the danger of using estrogen hormone during pregnancy."

J-56 Broster, LR
1 "A Form of Intersexuality"
British Medical Journal, January 21, 1956: 149-151

Review of a case in which infant whose mother had been treated with DES presented with an enlarged clitoris, no labia minora. The urethral opening was situated in front of the urogenital sinus. Laparotomy revealed tiny uterus.

J-59 Bongiovani, Alfred, M.D., DiGeorge, Angelo, M.D., Grumbach, Melvin, M.D.
1 "Masculinization of the Female Infant Associated with Estrogenic Therapy Alone During Gestation"
J. of Clin. End. and Met., 19:1004, 1959.

Four cases of masculinization of female infants - mothers all treated with stilbestrol alone during pregnancy.

- J-61 Marsh, Hadleigh, D.V.M.
1 "Urethral Occlusion in Lambs on Feed Containing Stilbestrol"
J. of the Am. Veterinary Med. Assoc. 139:9, Nov. 1961, p. 1019.

Lambs on feed containing stilbestrol developed urethral occlusion and enlargement of secondary sex glands.

- J-62 Travis, HF and Schnaible, PJ
2 "Effects of Diethylstilbestrol Fed Periodically During Gestation of Female Mink Upon Reproductive and Kit Performance"
Am. J. of Veterinary Research, 23:93, March 1962, p. 359.

Mink fed stilbestrol had an almost complete failure of breeding, including resorption of litters, lowered number of births, reduced kit weight, and increased kit mortality.

- J-62 Taussing, H.
2 "A Study of the German Outbreak of Phocomelia. The Thalidomide Syndrome."
JAMA, 180:1106, 1962.

First American publication regarding Thalidomide - should have alerted drug manufacturers to DES transplacental effects.

- J-62 Black, J.A., M.D., MRCP
3 "Effects on the Fetus and Newborn of Drugs Given During Pregnancy"
The Practitioner, 189:1129, July 1962, p. 99.

Various drugs affect the fetus as evidenced by animal and human findings. Stilbestrol alone or in combination is obviously contraindicated in early pregnancy.

- J-62 Moya F and Thorndike V.
4 "Passage of Drugs Across the Placenta"
Am. J. of OB & GYN, 84:1778, December 1, 1962.

The classic concept of the placenta as a simple, passive, semipermeable filter is no longer tenable in the light of the present knowledge . . . It seems unlikely that a living cell membrane could be absolutely impermeable to a given compound. On the contrary, it is now believed that any substance found in the maternal or fetal blood should be able to penetrate the placenta to some extent unless it is destroyed or altered during passage . . . These new concepts demand a reappraisal of the available information regarding the passage of foreign compounds across the placenta, with special emphasis on the kinetics of penetration.